AD_____

Award Number: W81XWH-05-1-0152

TITLE: A Novel Mechanism of Androgen Receptor Action

PRINCIPAL INVESTIGATOR: Charles T. Roberts, Jr., Ph.D.

CONTRACTING ORGANIZATION: Oregon Health and Science University
Portland OR 97239-0396

REPORT DATE: January 2008

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01-01-2008	Annual	15 Dec 06 – 14 Dec 07

4. TITLE AND SUBTITLE
A Novel Mechanism of Androgen Receptor Action

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-05-1-0152

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Charles T. Roberts, Jr., Ph.D.

E-Mail: robertsc@ohsu.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Oregon Health and Science University
Portland OR 97239-0396

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT This revised project has as its goal the characterization of a novel alternative product of the Her-2/neu/erbB2 protooncogene derived from intron retention. The product of this splicing mechanism, termed herstatin, is a secreted protein comprised of the N terminus of the Her-2 receptor tyrosine kinase and a unique, intron-encoded C terminus that allows binding to the other members of the EGFR/erbB family. This binding down-regulates erbB expression and inhibits EGF family signaling and cell proliferation. We have found that herstatin also binds to and down-regulates the IGF-I receptor (IGF-IR) and modulates IGF signal transduction. Herstatin is, therefore, a novel bifunctional inhibitor of erbB and IGF-IR signaling. Herstatin is expressed in prostate tissue and may represent a promising therapeutic target and biomarker in CaP. The work proposed in this project will assess the effect of herstatin on CaP cell phenotype, the expression of herstatin in CaP samples, and potential mechanisms of regulation of herstatin expression.

15. SUBJECT TERMS
IGF-I RECEPTOR, ERBB RECEPTORS, PROSTATE CANCER, METASTASIS

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	7	**19b. TELEPHONE NUMBER** *(include area code)*

Table of Contents

Introduction

This revised project focuses on the action of an alternative product of the HER-2/erbB2 gene, termed herstatin. Previous work has shown that this protein exhibits binding all members of the erbB family and down-regulation of EGFR/erbB1, HER-3/erbB3, and HER-4/erbB4. Herstatin also binds to the IGF-IR and the insulin receptor, and, in stably transfected MCF-7 breast cancer cells, down-regulates IGF-IR levels and action and up-regulates insulin receptor and some, but not all, insulin receptor-mediated signal transduction pathways. Our modified SOW addresses the potential of herstatin as a bi-functional inhibitor of both erbB and IGF-IR signaling in prostate cancer.

Body

In conjunction with the submission of the revised annual report for calendar year 2005, we proposed a modified Statement of Work to guide the research for the remainder of the project. The approved revised SOW comprised the three tasks outlined below.

Task 1. Characterize the effects of herstatin in prostate cancer cells.

a. *Generate lentivirus constructs encoding herstatin and infect androgen-sensitive (LNCaP) and insensitive (PC-3) CaP cell lines.*
b. *Analyze IGF-I signaling in acutely and long-term infected cells.*
c. *Assess effects of herstatin on proliferation, migration, and apoptosis.*

Task 2. Evaluate expression of herstatin in prostate cancer cell lines and clinical samples.

a. *Optimize quantitative RT-PCR amplicon probesets and determine herstatin vs HER2 mRNA levels in a series of CaP cell lines, including PC-3, PC-3/AR, LNCaP, DU-145, P69, M12, and 22rV.1.*
b. *Assay herstatin expression in normal prostate tissue and CaP biopsies (already obtained from OHSU Cancer Center tissue bank).*

Task 3. Investigate regulation of herstatin expression.

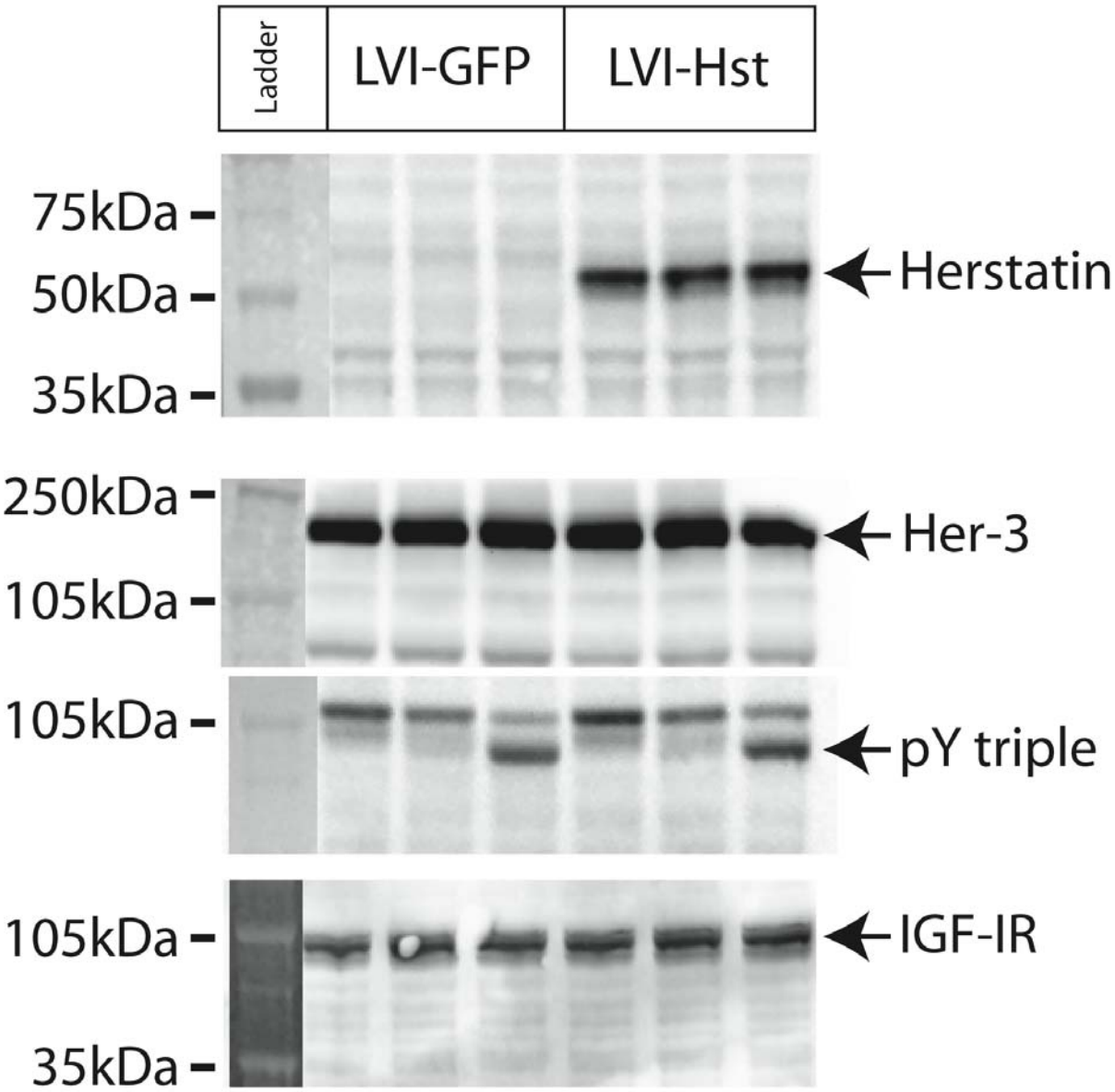
a. *Based upon preliminary data suggesting that heregulin increases herstatin expression in SKOV-3 cells, we will determine the effects of EGF system ligands on herstatin mRNA levels in CaP cell lines that express endogenous herstatin.*
b. *Utilizing minigene constructs that allow the assessment of intron 8 retention, evaluate differential intron retention activity in CaP cell lines and the effect of EGF ligands on minigene activity.*
c. *Determine the effect of WT1 (+KTS) on herstatin expression and minigene activity. This experiment is based upon a recent report that a particular splice variant of the WT1 tumor suppressor (which we have previously shown to be present in CaP [1] regulates intron retention and the translation of mRNAs containing retained introns [2].*

Progress in support of task 1.

We had previously designed and constructed a lentivirus vector encoding herstatin and generated active virus preparations. We have now infected MCF-7 cells with this preparation to produce cells that acutely express herstatin endogenously. We chose to do these initial infections in MCF-7 rather than CaP cell lines, since the original observations of herstatin effects were made in stably transfected MCF-7 cells. As shown in Figure 1, top panel, herstatin-encoding lentivirus-infected cells express easily detectable levels of herstatin in total cell lysates (right half of blot). The herstatin antibody is raised against the unique intron 8-encoded C terminus of herstatin, and is negative in control GFP lentivirus-infected cells (left half of blot). These levels are similar to those present in MCF-7 cells stably transfected with a plasmid-based vector described in the previous annual report (data not shown). The second and fourth panels of Figure 1 show the expression levels of Her-3 and IGF-IR in herstatin-lentivurus-infected cells. In distinct contrast to the effects noted previously in plasmid-transfected cells, acute expression of herstatin has no significant effect on the expression levels of these these cell-surface receptor tyrosine kinases. As shown in the third panel of the Figure, IGF-I activation of the IGF-IR as assessed by probing with an antibody directed against the phosphorylated activation loop in

the tyrosine kinase domain of the IGF-IR is equivalent in control and herstatin-expressing cells. Thus, the effects of long-term, plasmid-based herstatin expression differ from acute, lentivirus-based expression of similar levels of herstatin. We are now assessing the effects of long-term lentivirus-mediated herstatin expression on these same parameters.

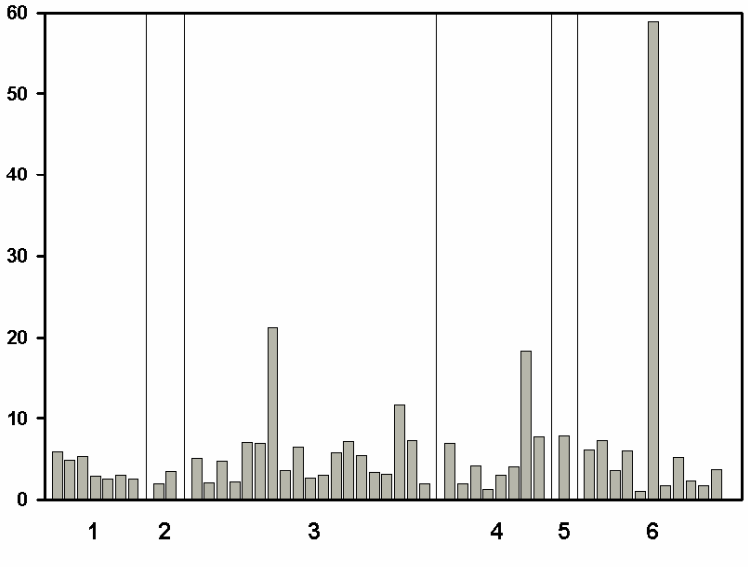
FIGURE 1. Expression of herstatin, Her-3, and IGF-IR in MCF-7 cells infected with a control, GFP-expressing (LVI-GFP) or herstatin-expressing (LVI-Hst) lentivirus. Also shown is activation of IGF-IR by IGF-I in control and herstatin-expressing cells (third panel down; compare lanes 3 and 6 (IGF-I-treated) to lanes 1 and 4 (basal)).



Progress in support of task 2.

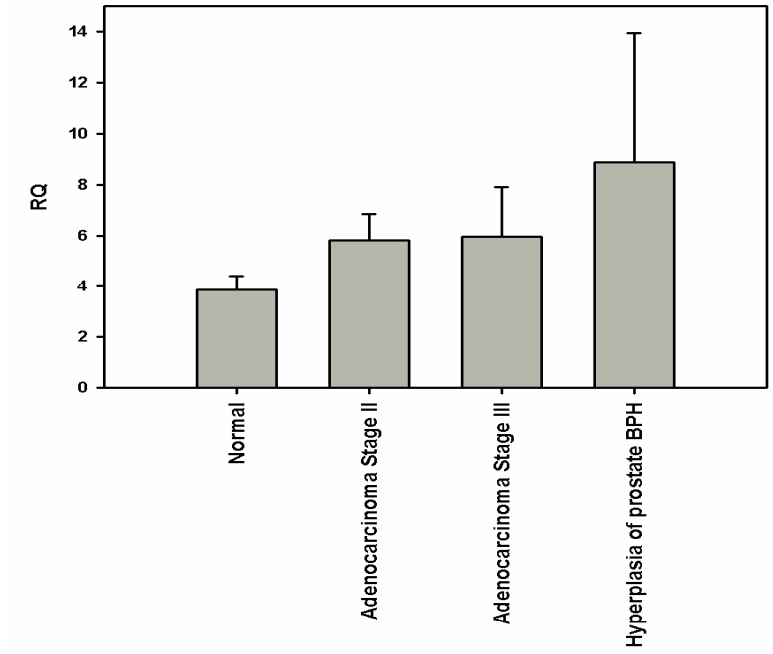
We had initially proposed to evaluate endogenous herstatin expression in various CaP cell lines and a limited number of clinical samples available from the OHSU Cancer Center tumor bank. As an alternative, we have exploited the commercial availability of an RNA array containing normalized total RNA samples from normal prostates, different CaP stages, and BPH. This array was analyzed by qRT-PCR (TaqMan technology) using a herstatin-specific amplicon consisting of primers in Her-2 exon 7 and intron 8. In control experiments, this probe gave a robust signal with RNA from MCF-7 cells transfected with a herstatin expression vector, but not with control cells. As shown in Figure 2, endogenous herstatin mRNA levels were similar in an extensive set of normal, CaP, and BPH samples, although there was some individual variability and some samples with particularly high relative expression. As illustrated in Figure 3, the average expression level was significantly higher in stage II and II CaP than in normal prostate, while the apparently higher level in BPH was influenced by the one high outlier. Thus, while herstatin mRNA is present in normal prostate, its expression levels is not decreased in CaP, as we would have predicted, but, rather, is somewhat increased.

FIGURE 2. Herstatin mRNA levels in normal prostate, CaP, bladder cancer, and BPH samples by qRT-PCR.



1 – Normal
2 – Adenocarcinoma of the prostate, stage I
3 – Adenocarcinoma of the prostate, stage II
4 – Adenocarcinoma of the prostate, stage III
5 – Carcinoma of the bladder, stage IV
6 – Hyperplasia of the prostate, BPH

FIGURE 3. Average Herstatin mRNA levels in normal prostate, CaP, and BPH samples by qRT-PCR.



Progress in support of task 3.

We are currently assessing the effect of erbB ligands on endogenous herstatin expression using the qRT-PCR approach validated in Figure 4 above.

Key research accomplishments

- Lentiviral expression of herstatin
- Demonstration of lack of effect of acute herstatin expression on presumptive targets
- Assessment of endogenous herstatin expression in clinical Cap samples and normal prostate controls

Reportable outcomes

None (manuscripts in preparation).

Conclusions

Our studies in the most recent year of funding have shown that acute expression of herstatin does not elicit the effects on erbB and IGF-IR expression or IGF-IR activation seen with long-term plasmid-based expression. Additionally, we have replicated evidence for herstatin expression at the mRNA level in normal prostate tissue, but did not find a predicted decrease in expression in CaP samples based upon the presumed antiproliferative effects of constitutive expression in breast cancer cells. The trend of increased expression in CaP may possibly reflect a compensatory response in CaP cells to prevent transformation. Subsequent analysis of the effects of long-term lentiviral expression may resolve this question.

References

1. Dong, G., Roopmathy, R., Vu, T., Hoffman, A. Rosenfeld, R., Roberts, C.T., Jr., Peehl, D.M., and Cohen, P. *J Clin Endocrinol Metab* (1997) **82**, 2198-2203.
2. Bor, Y., Swartz, J., Morrison, A., Rekosh, D., Ladomery, M., and Hammarskjöld, M.-L. (2006) *Genes & Dev* **20**, 1597-1608.

Appendices

None.

Supporting data.

NA